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研究課題名（和文）Additional genetic defects outside commonly deleted region in 22q11.2 deletion with psychosis; a prospect for identifying novel schizophrenia candidate genes

研究課題名（英文）Additional genetic defects outside commonly deleted region in 22q11.2 deletion with psychosis; a prospect for identifying novel schizophrenia candidate genes

研究代表者

ＳＨＡＢＥＥＳＨ　ＢＡＬＡＮ（SHABEESH, BALAN）

国立研究開発法人理化学研究所・脳科学総合研究センター・研究員

研究者番号：７０７２１５８８

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研究成果の概要（和文）：染色体22q11.2欠失は、統合失調症の最も大きなゲノムリスク要因であるが、欠失を伴った症例が100%統合失調症を発症するわけではない。このことは、22q11.2欠失とそれ以外のゲノム変異が合わさって統合失調症の発症に寄与すると考えられる。本研究では、① 22q11.2欠失を持ち統合失調症を発症した症例、および② 22q11.2欠失を持ちながら精神疾患を発症しなかった症例を同定し、２つのサンプルの全エクソンシークエンス解析を行った。その結果、KAT8, APOH, SNX31, EFCAB11, CLVS2の計５つの遺伝子を新たな統合失調症感受性遺伝子候補として同定した。

研究成果の概要（英文）：22q11.2 deletion is a prominent risk factor for developing schizophrenia, with varying penetrance. This suggests polygenic mechanisms requiring additional genomic variants for disease manifestation. We aimed to decipher the role of genetic defects outside the 22q11.2 region in increasing the risk for schizophrenia. We did whole exome sequencing on two subjects with 22q11.2 deletions; one with schizophrenia and other psychosis free. We identified 5 heterozygous variants in genes (3 frameshift: KAT8, APOH and SNX31 and 2 nonsense variants: EFCAB11 and CLVS2) outside the deletion region in 22q11.2 deletion patient with schizophrenia. Although these genes were relevant for neuronal function, interestingly, none of them were reported to be associated with any neurological/psychiatric phenotypes. These results will help in elucidating the variant dose and complex genetic architecture in schizophrenia. Role of these genes in manifestation of psychiatric phenotypes warrant future examination.

研究分野：　Genetics

キーワード：　22q11.2 deletion　Schizophrenia　SNX31　CLV2　EFCAB11　KAT8　APOH

１．研究開始当初の背景

Schizophrenia is a serious psychiatric disorder with high heritability and a worldwide lifetime risk of approximately 1% [1]. Although the exact etiology is unknown, genetic and environmental factors are known to play a significant role in disease pathogenesis. Although genetic contributions to schizophrenia risk are evidenced from twin studies, genome-wide association studies in schizophrenia have only identified genetic variants associated with a small effect on risk. A heterogeneous collection of rare structural variants are observed to contribute relatively large effects, in a modest proportion of schizophrenia cases, albeit with incomplete penetrance [2].

Among structural variants, 22q11.2 deletion is one of the highest risk factors for developing schizophrenia. This copy number variation is caused by hemizygous microdeletions at chromosome 22q11.2, and has a population prevalence of about 1 in 2,000 - 4,000 live births, with approximately one-fourth of carriers developing schizophrenia [3]. This chromosomal region is considered to be one of the main schizophrenia susceptibility loci, harboring several candidate genes for disease pathogenesis. Moreover, patients with 22q11.2 deletions who develop schizophrenia, are clinically and neurocognitively indistinguishable from patients with the idiopathic disorder, thus making deletion carriers an important model for understanding the pathophysiology of schizophrenia [4]. The clinical phenotype of 22q11.2 deletion subjects is characterized by varying expression and incomplete penetrance. The incomplete penetrance of schizophrenia in 22q11.2 deletion suggests polygenic mechanisms that require additional genomic variants outside of the commonly deleted region. Studies also have showed role of second hit mutations in conferring additional risks for psychosis. Thus we speculate role of additional genes in manifestation or modulation of the psychiatric phenotypes. The nature and role of these additive effects or epistatic interactions in the pathogenesis of schizophrenia are unknown. Thus characterizing these genetic networks may help in delineating disease pathology and understanding the relationship between schizophrenia and 22q11.2 deletions.

２．研究の目的

We proposed a comparative exome analysis in 22q11.2 deletion patients with and without manifestation of schizophrenia for identifying genetic variants outside commonly deleted region that confers additional risk for schizophrenia. These genes might contribute to the genetic network involved in the pathogenesis of schizophrenia; qualifying them as novel schizophrenia candidate genes.

３．研究の方法

This study aimed to decipher the role of genetic defects outside the 22q11.2 region in increasing the risk for schizophrenia. Patients with 22q11.2 deletion, for the study were clinically characterized for the presence/absence of schizophrenia by experienced psychiatrists. Based on the clinical characterization and inclusion criteria set we recruited two subjects with 22q11.2 deletion; one with schizophrenia (Subject A) and other psychosis free (Subject B). To confirm the 22q11.2 microdeletion, fluorescence in situ hybridization (FISH) with the TUPLE1 probe and array comparative genomic hybridization (aCGH) using NimbleGen Human CNV 3X720K v1.0 Array were performed.

Target enrichment for whole exome sequencing was performed using Agilent's SureSelect Human All Exon kit v2 (Agilent Technologies), and samples were sequenced using the Illumina HiSeq 2000 platform (Illumina Inc) and generated paired-end 100 bp reads. The resulting reads were aligned to the hg19 reference genome (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/) using Burrows-Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net/), and sequence-data analysis module CASAVA (Consensus Assessment of Sequence and Variation) v.1.8 (Illumina), which uses the Efficient Large-Scale Alignment of Nucleotide Databases (ELAND) algorithm. In addition, the variants were called using

CASAVA v.1.8 and Samtools (v.0.1.17) (http://samtools.sourceforge.net). Ensembl Variant Effect Predictor (VEP) (http://www.ensembl.org/info/docs/variation/vep/index.html) was used to annotate the variants by custom PERL scripts. The filtering of variants was performed using VarSifter (http://research.nhgri.nih.gov/software/VarSifter/). The identified variants were prioritized based on the following criteria: (a) present only in subject with 22q11.2 deletion manifesting schizophrenia; (b) novel, therefore not present in the National Center for Biotechnology Information dbSNP database (Build 137), 1000 Genomes Project or the Exome Variant Server of NHLBI GO Exome Sequencing Project (c) deemed functional, such as frameshift, stop-gain or non-synonymous mutations; (d) conserved on the basis of GERP (Genomic Evolutionary Rate Profiling) scores (>5) (e) predicted to be deleterious and damaging by PROVEAN and SIFT software. The identified variants were further validated and reconfirmed by Sanger sequencing.

4．研究成果

The FISH analysis and aCGH confirmed the 22q11.2 microdeletions, showing a 2.6 Mb hemizygous genomic deletion in both subjects (figure1).

Exome analyses yielded a total of 162,587,314 reads, of which 137,738,230

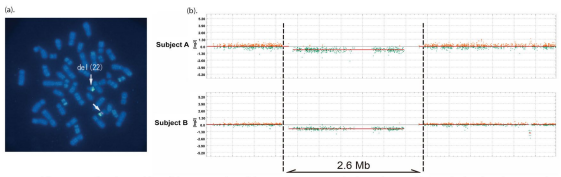

Figure1: (a) DNA FISH analysis showing deletion (b) CGH array analysis of chromosome 22 Upper panel: Subject A (22q11.2 deletion with schizophrenia), Lower panel: Subject B (22q11.2 deletion without schizophrenia). Both subjects show a 2.6 Mb hemizygous deletion at chromosome 22q11.2.

reads (84.72%) passed quality filters with a coverage across the target region of 83.49%, at 50x depth and 91.03% at 25x depth for subject A. For the subject B, 180,891,996 reads were obtained, with 152,498,040 reads (84.3%) passing quality filters, with coverage across a target region of 85.51% at 50x and 92.18% at 25x depths.

Variants were filtered further based on specified criteria, and for analysis, a higher priority was given to frameshift and stop gain or stop loss mutations. Additionally, based on higher GERP scores (> 5) that rank deleterious effects, we identified five

Table 1 List of variants identified solely in 22q11.2 deletion patient with schizophrenia (Person A)

Chromosome	Position (base pair)	Effect	Gene	Gene description	GERP score
14	90,263,658	Nonsense	*EFCAB11*	EF-hand calcium-binding domain 11	6.03
16	31,131,524	Frameshift	*KAT8*	K(lysine) acetyltransferase 8	5.83
17	64,219,861	Frameshift	*APOH*	Apolipoprotein H (beta-2-glycoprotein I)	5.59
8	101,642,574	Frameshift	*SNX31*	Sorting nexin 31	5.44
6	123,319,142	Nonsense	*CLVS2*	Clavesin 2	5.39

GERP, Genomic Evolutionary Rate Profiling.

heterozygous variants in genes (3 frameshift: *KAT8*, *APOH* and *SNX31* and 2 nonsense variants: *EFCAB11* and *CLVS2*) outside the deletion region in the 22q11.2 deletion patient with schizophrenia (Table1). All identified variants were verified by Sanger sequencing. Interestingly, none of the genes harboring these variants were previously reported to be associated with any neurological or psychiatric phenotypes, although they were relevant for neuronal function.

Information on specific variants identified from Subject A, could provide sound putative candidate genes for further schizophrenia analysis. One candidate gene that harbored a frameshift mutation was *SNX31* (Sorting Nexin 31) which codes for a family of SNX proteins, containing a conserved PX (or phagocyte oxidase homology) domain that targets SNX proteins to endosomes [5]. This protein family is involved in glutamate receptor recycling and is thought to contribute for the pathogenesis of the schizophrenia [6]. In addition, a nonsense mutation was observed in the *CLV2* gene (clavesin 2) which is involved in modulating neuron-specific regulation of late endosome/lysosome morphology [7]. It is plausible that both of these genes may potentially increase schizophrenia risk due to their roles in neuronal specificity and vesicular transport.

Another gene identified from Subject A, carries a non-sense mutation and codes for an EF-hand calcium-binding domain-containing protein 11 (EFCAB11) which may function as sensor proteins, buffer proteins or Ca2+-stabilizing proteins. Since calcium signaling plays an important role in the regulation of cell metabolism, gene expression, cytoskeleton dynamics, cell cycle, cell death, neurotransmission and signal transduction processes, it is not implausible to speculate EFCAB11 advancing psychiatric phenotypes.

Two frameshift mutations, were detected in the genes for K(lysine) acetyltransferase 8 (KAT8) and

apolipoprotein H (beta-2-glycoprotein I) (APOH). The KAT8 gene is a member of the MYST histone acetylase protein family, which can mediate epigenetic changes through histone modifications. The APOH gene is described as a major antigenic target for antiphospholipid antibodies, leading to antiphospholipid syndrome [8]. Memory alterations, cognitive impairment, mood disorders and psychosis are known to precede the onset of primary antiphospholipid syndrome [9]. Therefore, the role of *APOH* mutations in neuropsychiatric phenotypes warrants further study.

No novel mutations in the 22q11.2-hemizygous region were observed in 22q11.2 deletion patient with schizophrenia. However, in Subject B, a novel, deleterious, nonsynonymous mutation (D203N) was observed in the synaptosomal-associated protein, 29kDa (SNAP29). Rare variants of SNAP29 were recently identified in a series of patients with 22q11.2 deletion syndrome, unmasking autosomal recessive conditions that resulted in atypical phenotypes such as cerebral dysgenesis, neuropathy, ichthyosis and keratoderma [10]. However none of these atypical phenotypes were present in Subject B.

The novel genetic variants related to neuronal function exclusively in the patient with schizophrenia will pave the way towards a more complete understanding of variant dose and genetic architecture in schizophrenia. Role of these genes in manifesting/modulating psychiatric phenotypes warrant future examination.

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５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）
〔雑誌論文〕（計 1 件）

1. **Balan, S.**, Iwayama, Y., Toyota, T., Maekawa, M., Toyoshima, M. & Yoshikawa, T. (2014). Exome comparison of two 22q11.2 deletion carriers reveals potential schizophrenia-associated novel variants. ***The British journal of psychiatry***, 204, 398-399.**査読有り**

６．研究組織
(1)研究代表者 Shabeesh Balan
（シャビーシュバラン）
国立研究開発法人理化学研究所・脳科学総研究センター・研究員
研究者番号：70721588